Final for FREP 11-0453

A. Project Information.

Period: January 2011 to July 2015

Title: Exploring the Potential of Transgenic Crops for Improved Fertilizer Use Efficiency

Project Leaders: Charles A. Sanchez, Professor, University of Arizona, Maricopa Agricultural Center, 37860 W Smith Enke Rd, Maricopa, AZ 85138, phone 928-941-2090, e-mail sanchez@ag.arizona.edu

Roberto Gaxiola, Assistant Professor, School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287-4501, (480) 965-3337, e-mail roberto.gaxiola@asu.edu

B. Objectives:

The objectives of this project are to evaluate and demonstrate the potential for using AVP1 modified plants for improved nutrient use efficiency under desert cropping systems. Studies will include potato, lettuce, and cotton. We have already modified potato, cotton and romaine lettuce. As part of this project we seek to modify iceberg lettuce, a significant user of fertilizer in the western United States.

C. Abstract:

Crops produced in the desert receive large annual applications of nitrogen (N) and phosphorus (P) fertilizer. However, declining energy supplies and P mineral reserves, erratic fertilizer costs, and concerns about water pollution, has created incentives for improved efficiency. While we have developed management practices such as soil and plant tissue testing and improved fertilizer placement and timing, the possibility of genetic modifications to crops for improved fertilizer use efficiency has received little attention. More recently, it has been shown that over-expression of type I H^+ -pyrophosphatase AVP1 (AVP, *Arabidopsis* vacuolar pyrophosphatase) can enhance nutrient acquisition by crops. AVP1 over-expressing (AVP1 OX) tomato (*Lycopersicon lycopersicum* L.) plants produced more shoot and root biomass than controls when grown under phosphate and nitrate limitations and accumulate more potassium in all conditions tested. The objective of this project is to evaluate the potential for using AVP1 modified crops for improved nutrient use efficiency under desert cropping systems. The data show AVP1-OX romaine lettuce outperformed conventional lettuce at all levels of N and P in 2011-2012. For greenhouse tomato AVP1-OX cultivars only out performed conventional cultivars when P was limiting. In 2013, crop responses were often limited by high residual fertility and heat stress. Additional field studies with cotton in 2015 showed the AVP modified cotton outperformed the conventional cotton at all N rates. There was no response to P in 2015

and cotton in the desert rarely responds to P. Field testing of AVP modified iceberg lettuce cultivars in 2016 showed that they outperformed their conventional counterparts, especially at low P.

D. Introduction

Crops produced in the desert receive large annual applications of nitrogen (N) and phosphorus (P) fertilizers. Amounts of N applied range from 200 to 400 kg/ha and crop recoveries are generally less than 50%. There are numerous possible fates of fertilizer applied N in addition to the desired outcome of crop uptake. The urea and ammonium components of the N fertilizer might be lost through ammonia volatilization. The nitrate-N might be lost to leaching with irrigation water below the crop root zone possibly impairing surface and ground water. Nitrate might also be lost as N_2 and N_2O gasses via denitrification processes affecting air quality and climate. Furthermore, all forms of N might be immobilized into the organic soil fraction by the soil microbial population where availability to the crop is delayed. Nitrogen fertilizer production depends on natural gas availability and prices.

Amounts of P applied to crop production systems often approach and exceed 200 kg P/ha and crop recoveries of P fertilizers are generally less than 20%. While much of the added P is converted to insoluble forms in the calcareous soils of the region, some of it is potentially carried off in runoff and drainage water into receiving surface waters having adverse ecological effects. Further, erratic fertilizer pricing over the past several years has created incentives for improved efficiency. Approximately three years ago, the costs of mono-ammonium phosphate (MAP), a formulation widely used for desert vegetable production, exceeded $1,200.0 per ton. Although costs have since declined, rapid increases are anticipated as the world economy recovers and resource demand in the developing world regains momentum. In addition, world P reserves are rapidly declining and there is concern that a shortage of P fertilizers will ultimately compromise world food production.

Over the past two decades, researchers with the Universities of Arizona and California have developed strategies for efficient nutrient management. For N, these practices include fertilizer timing or controlled release fertilizers, pre-sidedress plant and soil testing, and improved irrigation management. For P, these practices include soil test based fertilizer recommendations and exploitation of innovative placement technologies. However, the possibility of genetic modifications to commercial crops for improved fertilizer use efficiency has received little attention. A high fertilizer use crop such as lettuce shows very little variation in response to fertilizer, among commercial cultivars currently used.

More recently, it has been shown that that over-expression of type I H^+ -pyrophosphatase AVP1 (AVP, *Arabidopsis* vacuolar pyrophosphatase) contribute positively to many energetic plant processes including general growth, nutrient acquisition, and stress response. This genetic modification enhances nutrient uptake by affecting the abundance and activity of the plasma membrane H^+-ATPase in a manner that correlates with apoplastic pH alterations and rhizosphere acidification. Rhizosphere acidification is a central mechanism for plant mineral nutrition since it contributes to nutrient solubility and the plasma membrane proton motive force. Preliminary data we have collected show the potential for yields of AVP1 romaine lettuce to be maximized with less P fertilizer than that required for conventional cultivars.

The objective of this project is to evaluate the potential for using AVP1 modified plants for improved nutrient use efficiency under desert cropping systems. Studies will include potato, lettuce, and cotton. Field studies will include N and P rate studies and comparisons of AVP1 modified cultivars and conventional cultivar counterparts. These studies will be conducted at university research centers in Yuma and Maricopa AZ, and in Holtville and Thermal, CA due to existing restrictions concerning GMOs and field production. Additionally, we seek to genetically modify iceberg lettuce for AVP1 expression and perform preliminary evaluations of these modifications.

E. Work Description.

Task 1

During 2012 we completed subtask 1a by transforming two cultivars of iceberg lettuce for AVP1-OX. We have grown out T1 seed from at least five transgenic events from each of these modified cultivars in the greenhouse. We are now screening (subtask 1b) this germplasim using our selectable marker (Kanamycin). This task has took longer than anticipated due to delays in methodology development. We have screened T2 seed. However, we are now collecting T3 seed for these iceberg lettuce cultivars at present.

Task 2

We initiated some small field studies with AVP1 modified potato in spring 2012 (Task 2a). Unfortunately, for potato, it seems this transformation produces an excess number of potatoes, none of which makes acceptable size. We will try once more in 2013. However, at the prospect that potato will not work, we have initiated studies with AVP1-OX tomato.

We had transformed the cultivar “Money Maker” which is a greenhouse cultivar. Thus, our initial experiment was conducted in the greenhouse. Three gallon pots were filled with a 50:50 blend of Grande (fine-loamy, mixed, hyperthermic, Typic Natriargid (reclaimed)) and silica sand to facilitate automatic irrigation without soil cracking. The pre-plant Olsen P test was < 5 mg/kg. All pots received 1.5 g of N as a controlled release N fertilizer (ESN distributed by Agrium Advanced Technologies) so that N would not be limiting. The experimental P rates were 0, 0.125, 0.25, 0.5, and 1.0 g P/pot applied as triple superphoshate. Tomato transplants grown in greenhouse trays were transplanted one plant per pot.

We are increased our cotton seed under greenhouse conditions (subtask 2b). Cotton had proved difficult for APHIS permitting as well as to protect our own germplasm from contamination from commercial GMOs. Essentially all of the cotton planted commercially in Arizona southern California is double GM modified for BT protection and Glyphosate tolerance and we do not wish to be caught in a legal dispute if these traits cross into our lines. Thus all our seed increase is being conducted in the greenhouse. We proceeded to the field in 2013 once we had sufficient seed and negotiated an acceptable field protocol with APHIS.

Task 3

Several field and greenhouse studies with N and P were conducted with AVP1-OX romaine lettuce in 202-2013. In most cases we conducted backup greenhouse studies for more intensive sampling since all plant and soil material are regulated articles and the logistics of transporting large numbers of samples from the field to the laboratory and subsequent disposal is onerous (double leak proof containment), laborious, and often cost prohibitive. The experimental design for all greenhouse experiments was randomized complete block with four replications. The treatment design for the field experiments was split plot where fertilizer rate was the main plot and cultivar the subplots.

Statistical analyses were performed using SAS where responses to N or P were evaluated by trend analysis and differences among cultivars by least significant difference.

N Studies with AVP-OX Romaine lettuce in 2011-2012

Surface soil mapped as Casa Grande (fine-loamy, mixed, hyperthermic,Typic Natriargid (reclaimed)) was collected at the Maricopa Agricultural Center, sieved, and 1.6 kg were weighed into 15 cm diameter pots. The pre-plant nitrate-N test for this soil was 50 mg kg^{-1}. All pots received 0.34 g P as mono-calcium phosphate so that P would not be limiting. The treatments included 'Conquistador' (also referred to as conventional or WT), AVP1D2, and AVP1D6 romaine lettuce and N rates. The AVP-OX lines are transformed 'Conquistador'. The total seasonal rates of N were 0, 0.1, 0.2, 0.4, and 0.8 g N per pot. The N was applied as a potassium nitrate solution in eight split applications to achieve these seasonal total rates. The potassium nitrate solution was labeled with 10 atom percent ^{15}N.

Lettuce seedlings (one-leaf stage) were transplanted into the pre-watered pots. The first N fertilization occurred 5 days after transplanting and continued twice weekly through harvest. These plants were grown to the eight-leaf stage and harvested by cutting the above ground plant at the soil surface. Total leaf area was measured using a LiChor area meter (LI 3100 C), and fresh and dry weights were determined as described above. Total N and ^{15}N percent were determined by combustion mass spectroscopy.

A field study was planted in the same field where we collected soil for the greenhouse experiment. The entire plot area received 125 kg P ha^{-1} as mono-ammonium phosphate which is the common practice for low P testing soils. Thus, the entire plot area also received 54 kg N ha^{-1} with the pre-plant phosphate fertilizer. The N rate treatments of 0, 50, 100, 150 and 200 kg N ha^{-}

[1] (not including that applied with the pre-plant phosphate fertilizer) were applied pre-plant as a polymer coated urea controlled release N product (ESN distributed by Agrium Advanced Technologies). All N and P fertilizers were roto-mulched into the beds. Individual main plots were 25 m^2 and the experimental design was randomized complete block with four replications.

Lettuce cultivars were seeded in elevated double row beds on 1 m centers with a hand planter (Jang JP1 Clean Seeder) and thinned by hoe at the four-leaf stage to approximately 71,000 plants per hectare. The stands were established by sprinkler irrigation. After establishment, all required irrigations were applied by level (no slope) furrows. Lettuce was harvested at maturity by cutting and weighing all plants from 3 m of double row beds. Marketable yield was determined after grading using standard practices.

P Studies 2011-2012

We conducted four separate greenhouse studies to evaluate the response of conventional and AVP1-OX romaine lettuce to P. For the first experiment we used a Superstition sand (sandy, mixed, hyperthermic Typic calciorthid), for the second and third experiments we used Casa Grande (fine-loamy, mixed, hyperthermic,Typic Natriargid (reclaimed)). These three experiments were watered as needed by hand. For the fourth experiment we used a 50:50 mix of Casa Grande and silica sand because we used an automatic irrigation system and we did not want soil cracking to compromise irrigations. In all experiments 1.6 kg were weighed into 15 cm diameter pots. The pre-plant Olsen soil test levels were < 7 mg/kg in all these greenhouse studies.

All pots received 0.8 g N so that it was not limiting. In experiments 1 through 3 we used split applications of potassium nitrate. In experiment 4, we used a controlled release fertilizer (ESN). The P rates were 0, 0.04, 0.08, 0.17, and 0.34 g P/pot applied as triple superphosphate. All P fertilizers were applied pre-plant. Lettuce seedlings (one-leaf stage) were transplanted into the pre-watered pots and whole above-ground plants were harvested at the eight-leaf stage.

Three field studies were conducted in 2012-2013 on a field mapped as Casa Grande (fine-loamy, mixed, hyperthermic,Typic Natriargid (reclaimed)). However, for purposes of discussion we included another field study conducted in 2011 on an Indio silty clay loam (mixed hyperthermic Typic Torrifluvent) before we had FREP funding. The study conducted in 2011 before FREP funding had a pre-plant Olsen P test of 25 mg/kg. The P source in the study was mono-ammonium phosphate because this is what growers primarily use in the desert for vegetables. This study was direct seeded using an air-planter.

The first study conducted in 2012 with FREP funding was with transplants. The P source in this study was triple superphoshate. The transplants were produced in a greenhouse and set in the field with a mechanical transplanter. The study had a pre-plant Olsen P test of <5 mg/kg. The second study in 2012 had the same P source but was direct seeded using a hand planter (Jang JP1 Clean Seeder). This study had a pre-plant Olsen P test of <5 mg/kg. For the third study in 2012, we again used mono-ammonium phosphate as the P source. The study also had a pre-plant Olsen P test of <5 mg/kg. The P rates in all four field experiments were 0, 25, 50, 75, and 100 kg P/ha. All P fertilizer was applied pre-plant onto the beds and power-mulched into the soil. N was applied to the entire plot area so that it would not limit production. A total N application of 200 kg/ha was applied as a combination of sidedress and water run applications. The lettuce was thinned by hoe at the four-leaf stage to approximately 71,000 plants per hectare. The stands were established by sprinkler irrigation. After establishment, all required irrigations were applied by level (no slope) furrows. Lettuce was harvested at maturity by cutting and weighing all plants from 3 m of double row beds. Marketable yield was determined after grading using standard practices.

In spring 2013 we initiated field fertility trials with cotton. For the N study, the N rates were 0, 75, 150, and 225 kg N/ha. For the P study, the P rates were 0, 25, 50, and 75 kg/ha. These studies included wild-type “Coker” and two transgenic lines of this cultivar (line 68 and 86). Both experiments were planted by hand April, 4 2013. The experiment design was randomized complete block with 4 replications. These experiments were harvested by hand November 21 and 28, 2013.

These experiments were repeated in 2014.

Tasks 4

N and water studies with romaine lettuce

Two studies were conducted evaluating the response of AVP-OX romaine lettuce to N and water. The studies were factorial combinations of water (60, 100, and 140% ET) and N (60, 120, and 180 kg N/ha). These studies were conducted under drip irrigation. The system was designed with nine manifolds and nine mains to deliver the various treatment combinations to plots in a randomized complete block design. The irrigation were based on AZMET generated ET measurements.

We seeded romaine studies on irrigation and N fertilization on November 5, and December 22. The crops were established by sprinkler irrigation, After stand establishment all irrigation and fertilizations were applied through the drip system. The first and second lettuce romaine studies were harvested on April 4 and April 26, respectively.

P studies with potato

A second potato experiment was planted February 26, 2013. The P rates were 0, 50, and 100 kg P/ha. The study included non-transgenic “Desirae” and three transgenic transformations of this cultivar. The experiments was irrigated and fertilized with N (200 kg N/ha) by buried drip irrigation. These potatoes were harvested June 17, 2013.

1st P study with tomato

Tomato “Money Maker” and “AVP-OX Money Maker” were seeded in the greenhouse. The P rates in this experiment were 0, 25, 50, and 100 kg P/ha. The tomato transplants were set in the field March 23, 2013. The experiments was irrigated and fertilized with N (200 kg N/ha) by buried drip irrigation. Tomato yields were harvested July 3, 2013 to July 17, 2013.

2nd P study with tomato

We did a second study evaluating 7 AVP-OX back crossed tomato. These were started in the greenhouse as above and transplanted March 23, 2013. The P rates for this study were 0, 40, and 100 kg P/ha. The experiments was irrigated and fertilized with N (200 kg N/ha) by buried drip irrigation. Tomato yields were harvested July 3, 2013 to July 17, 2013.

Transformation of corn

The Ad Hoc and TASC reviewers suggested we include agronomic crops in our evaluations. Although we included cotton, we deiced to also include corn. The corn was transformed at the Plant Transformation facility at Iowa State University. The seed from several transgenic events were be planted in the greenhouse and field. The seed was harvested December 10.

2015 Cotton

N and P rate trails with AVP and conventional cotton were initiated in April. These will be harvested in late September.

Tasks 5 and 6

Due to delays in screening and increasing seed for iceberg lettuce we did not have inbred at the end of the spring 2015 growing season. Iceberg lettuce field trails were not conducted during the spring of 2016. The cultivars ‘Winterset, Winterhaven, and their AVP modified counterparts were grown under no and adequate P. Seed limitations resitrcted the number of treatments.

F. Data/Results

Task 1

The development of inbred AVP-OX iceberg lettuce lines is on-going.

Task 2

Overall, the AVP1-OX tomato showed more rapid growth and development compared to the conventional type across all P rates (Figure 1). The yield data are interesting where early production was higher for AVP1-OX tomato compared to the conventional at all P rates (Figure 2). However, as harvests continued, cumulative yields at higher P rates were greater for the conventional compared to the AVP1-OX tomato. For example, maximum yields of AVP1-OX tomato were approximately 1200 g fresh fruit per pot at 0.125 g P/pot and were significantly greater than the conventional at this P rate. However, AVP1-OX tomato showed no further increase to higher P rates while conventional did, ultimately out-yielding the AVP1-OX tomato at P rates greater than 0.125 g P/pot.

Task 3

N Studies

Lettuce above ground dry matter and leaf area significantly ($P<0.01$) increased by N rate (Figures 3 and 4). Furthermore, cultivar effects were also statistically significant where AVP1D6 outperformed AVP1D2 which in turn outperformed the conventional conquistador. Leaching of N in this greenhouse experiment was high due to the frequent watering required to maintain plant vigor in the warm greenhouse. Total accumulation of N and fertilizer N significantly ($P<0.01$) increased with N rate and was statistically ($P<0.05$) higher in AVP1-OX plants compared to conventional Conquistador (Table 1). On average over 90% of the N accumulated in the plant was derived from the applied fertilizer.

Romaine lettuce showed a significant ($P<0.05$) quadratic response in the field where marketable yields were essentially maximized to the first N rate (50 kg N ha^{-1}) and N rates beyond 100 kg N ha^{-1} reduced yields (Figure 5). Cultivar response was highly significant ($P<0.01$) where both AVP1D2 and AVP1D6 produced more marketable yield across all N rates compared to unmodified Conquistador.

P Studies

In all greenhouse experiments, lettuce dry matter yields increased to P rate (Table 2 and Figure 6). Further, the AVP-OX lettuce consistently outperformed the conventional "Conquistador".

In all field experiments there was response to P and there were significant differences among cultivars (Table 3). In experiment 1 (2011), response to P was small as pre-plant soil test were 25 mg/kg. Lettuce typically does show some response to P fertilizer up to Olsen soil test P of 35 mg/kg and there was a modest response to P for the conventional cultivar (Figure 7). However, yields of the AVP1-OX romaine lettuce were near maximum and significantly higher than the conventional cultivar when no P was added suggesting these plants can take up P that the unmodified cultivar could not.

In the experiments conducted on a low P testing soil (experiments 2 through 4), marketable yields were generally higher for AVP1-OX lettuce across most P rates (Table 3 and Figure 8 and 8). Interestingly, marketable yields for AVP1D6 lettuce were generally a little higher compared to AVP1D2 lettuce, although usually not statistically significant. This is in contrast to the greenhouse experiments where dry matter was generally higher for AVP1D2 compared to AVP1D6, although not statistically significant. We have other data showing greater root growth for AVP1D6 compared to AVP1D2 and perhaps in the greenhouse the pots restricted root growth.

N and P with Cotton

The first cotton experiment was harvested November 14 and 28. As noted previously, the results of this experiment should be interpreted with caution since seed was limited and plots were only 5 ft long. Cotton yields increased by N but not P (Table 5 and 6). Interestingly, in the N experiment, WT outperformed the other cultivars but in the P experiment line 68 outperformed the other cultivars. The observation that 68 in a stronger cultivar has been noted in previous studies.

In 2014, we repeated these studies with WT and line 68 (Figures 9 and 10). In these studies there were significant interactions. In these experiment cotton responded and 68 generally outperformed WT. IN the P fertilizer experiment, 68 outperformed WT at low P rates but WT outperformed 68 at high P rates.

In 2015, the AVP cotton outperformed the conventional across all N rates (Table 10). There was no response to P in 2015 (data not shown).

Task 4

Water and N Experiment with AVP-OX romaine lettuce

The yield responses of conventional and AVP-OX romaine lettuce to water and N are shown in Tables 6 and 7. Overall, there was a large response to irrigation. Unfortunately, this soil had high residual N levels and there were no positive responses to N fertilization. In the first experiment there was no response to N at all. In the second experiment, N fertilization reduced yields, where the reduction was more pronounced when N was limiting. There were no significant differences in conventional and AVP-OX romaine lettuce. Previous reports had reported enhanced drought tolerance to AVP-OX. We did not observe that in this experiment. In experiments conducted in 2011-2012, AVP-OX showed improved N utilization efficiency. In this experiment, it appears N was not limiting so we could not validate this response in 2012-2013

P studies with potato

The response of conventional and AVP-OX potato to P is shown in Table 8. There were no response to P. The initial P soil test were medium which may have limited the response because potato are not as responsive to P as lettuce. There were no significant differences in yields of conventional and AVP-OX potato. It has been reported that potato largely relies on symplastic transport nutrient pathways which may limited response to AVP-OX. We will not continue work with potato in year 3.

P studies with tomato

The response of conventional and AVP-OX tomato to P is shown in Table 9. There were no yield response to P. The initial P soil test were medium which may have limited the response because tomato, like potato, are not as responsive to P as lettuce. There were no significant differences in yields of conventional and AVP-OX tomato. At harvest day time, day time high temperatures approached 115 F and, heat stress seemed to limit yields of the "Money Maker" cultivar. Interestingly we also evaluated some back crossed AVP1-OX and two of these back crosses performed exceptionally well. We will repeat studies with AVP-OX tomato in 2014.

P studies with transformed iceberg lettuce

The results for iceberg lettuce are shown in Figure 11. The AVP transformed lines outperformed their conventional counterparts, particularly when P was limiting.

G. Discussion and Conclusions

Overall, the large number of greenhouse and field experiments conducted with lettuce show that AVP1-OX shows great promise as a tool for improving nutrient use efficiency in the desert. AVP1-OX lettuce consistently outperformed conventional lettuce at most N and P rates in greenhouse and field experiments in 2011-2012. Despite observation of improved N utilization efficiency for N in romaine lettuce in 2011-2012 we did not see this in 2012-2013, probably due to high residual N levels in the soil.

The inconsistent results with tomato were surprising. While AVP1-OX lettuce outperformed conventional lettuce at all P fertilizer levels, AVP1-OX tomato only outperformed conventional tomato at suboptimal P levels in the greenhouse experiments. Yields of conventional and AVP-OX in P field experiments were similar and generally low due to heat stress. Perhaps the differences observed between lettuce and tomatoes are associated with a crop harvested in a vegetative state such as lettuce, and a crop harvested at a reproductive state, such as tomato. Interestingly, cotton was as inconsistent as tomato.

There were no significant differences in yields of conventional and AVP-OX potato. It has been reported that potato largely relies on symplastic transport nutrient pathways which may have limited response to AVP-OX.

Overall, the large number of greenhouse and field experiments conducted with lettuce show that AVP1-OX shows great promise as a tool for improving nutrient use efficiency in the desert. Results with fruiting crops are less consistent.

H. Project Impacts

This project provided proof in that there was a potential genetic path to improved nutrient use efficiency if realistic production settings. Results with lettuce were consistent for N and P.

Results with fruiting crops has been less consistent. Impact is limited by the regulatory requirements of developing GMO crops, particularly food crops. However, inspired by this project and with funding from other sources we have begun to explore a non GMO path. We have begun evaluating recombinant inbred lines (RILs) of lettuce and we have found significant variation in rhizosphere acidification, and N and P uptake across nutritional regimes. We are currently pursuing quantitative trait loci analysis (QTL).

I. Outreach

Seeking a Genetic Path for Improved Nutrient Use Efficiency. 2012 SW Ag Summit. March 8, 2012. Approximately 45 producer and crop advisors attended this meeting. There was substantial interest and questions had to be cut off. A few followed me outside for follow up information. This was a very effective venue.

Phosphorus management in lettuce. July 16 2012. Approximately 15 growers attended this meeting of the Arizona Iceberg Lettuce Research Council. There was a small crowd but a lot of questions.

Exploring Variable Rate Phosphorus Application Methods in Vegetables. Preseason Vegetable workshop. August 30, 2013. Approximately 69 producer and crop advisors attended this meeting. In this venue I summarized AVP1 work and introduced new FREP project on variable rate. A large audience and several questions.

Seeking Agronomic Solutions to Global P Challenges. November 26, 2012. An invited presentation in Tucson to a mixed audience of scientist, students, crops advisors, and producers. There were about 24 people present in the audience. Most of the questions came from the academic audience. This was a less effective outreach venue than others.

We also participated a field day in April at the Maricopa Agricultural Center where attendees visited the FREP plots. There were approximately 24 producers and crop advisors at this field day. There was enthusiastic participation and a lot of questions.

We presented this data at the Irrigation and Nutrient Management meeting in Salinas CA on February 12, 2014. There were approximately 78 producers and crop advisors.

We summarized are findings at SW Ag Summit in Yuma on February 27, 2014. There were 45 producers and crop advisors and numerous questions.

We presented this data at the Desert Ag conference on May 18, 2014 in Chandler Arizona. There were 34 producers and crops advisors and numerous questions.

Our FREP AVP plots were also visited as a stop on field tours at the Maricopa Ag. Center.

J. Factsheet Database Template

1. Project Title: Exploring the Potential of Transgenic Crops for Improved Fertilizer Use Efficiency

2. Grant Agreement Number FREP 11-0453

3. Project Leaders

Charles A. Sanchez, Professor, University of Arizona, Maricopa Agricultural Center, 37860 W Smith Enke Rd, Maricopa, AZ 85138, phone 928-941-2090, e-mail sanchez@ag.arizona.edu

Roberto Gaxiola, Assistant Professor, School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287-4501, (480) 965-3337, e-mail roberto.gaxiola@asu.edu

4. Start/end date January 2011 through July 2015

5. Project Location Low Desert Region of Southwestern US

6. Counties Imperial County, CA, Yuma County AZ, and Pinal County AZ

7. Highlights

AVP1 modified lettuce showed improved N and P utilization efficiencies under greenhouse and field production scenarios.

Results with fruiting crops such as tomato and cotton were less consistent.

We have initiated work to find a non-GMO genetic path toward improved N and P utilization efficiency.

8. Introduction

Crops produced in the desert receive large annual applications of nitrogen (N) and phosphorus (P) fertilizers. Amounts of N applied range from 200 to 400 kg/ha and crop recoveries are generally less than 50%. There are numerous possible fates of fertilizer applied N in addition to the desired outcome of crop uptake. The urea and ammonium components of the N fertilizer might be lost through ammonia volatilization. The nitrate-N might be lost to leaching with irrigation water below the crop root zone possibly impairing surface and ground water. Nitrate might also be lost as N_2 and N_2O gasses via denitrification processes affecting air quality and climate. Furthermore, all forms of N might be immobilized into the organic soil fraction by the soil microbial population where availability to the crop is delayed.

Amounts of P applied to crop production systems often approach and exceed 200 kg P/ha and crop recoveries of P fertilizers are generally less than 20%. While much of the added P is converted to insoluble forms in the calcareous soils of the region, some of it is potentially carried off in runoff and drainage water into receiving surface waters having adverse ecological effects.

Over the past two decades, researchers with the Universities of Arizona and California have developed strategies for efficient nutrient management. For N, these practices include fertilizer timing or controlled release fertilizers, pre-sidedress plant and soil testing, and improved irrigation management. For P, these practices include soil test based fertilizer recommendations and exploitation of innovative placement technologies. However, the possibility of genetic modifications to commercial crops for improved fertilizer use efficiency has received little attention. A high fertilizer use crop such as lettuce shows very little variation in response to fertilizer, among commercial cultivars currently used.

More recently, it has been shown that that over-expression of type I H^+ -pyrophosphatase AVP1 (AVP, *Arabidopsis* vacuolar pyrophosphatase) contribute positively to many energetic plant processes including general growth, nutrient acquisition, and stress response. This genetic modification enhances nutrient uptake by affecting the abundance and activity of the plasma membrane H^+-ATPase in a manner that correlates with apoplastic pH alterations and rhizosphere acidification. Rhizosphere acidification is a central mechanism for plant mineral nutrition since it contributes to nutrient solubility and the plasma membrane proton motive force. Preliminary data we have collected show the potential for yields of AVP1 romaine lettuce to be maximized with less P fertilizer than that required for conventional cultivars.

The objective of this project was evaluate the potential for using AVP1 modified plants for improved nutrient use efficiency under desert cropping systems.

9. Methods/Management

We utilized romaine lettuce, potato, cotton, and tomato previously transformed for pyrophospatase overexpression (AVP1). As part of this project we transformed iceberg lettuce and corn. Replicated greenhouse and field experiments were conducted across a range of N and P application rates.

10. Findings

Overall, the large number of greenhouse and field experiments conducted with lettuce show that AVP1-OX shows great promise as a tool for improving nutrient use efficiency in the desert. AVP1-OX lettuce consistently outperformed conventional lettuce at most N and P rates in greenhouse and field experiments. Results with fruiting crops are less consistent. With funding from other sources we have begun to explore a non GMO path toward improved N and P utilization efficiency by lettuce. We have begun evaluating recombinant inbred lines (RILs) of lettuce and we have found significant variation in rhizosphere acidification, and N and P uptake across nutritional regimes.

Figure 1. Image demonstrating tomato growth to increasing P rate (left to right) and conventional (front) and AVP-OX (back) tomato.

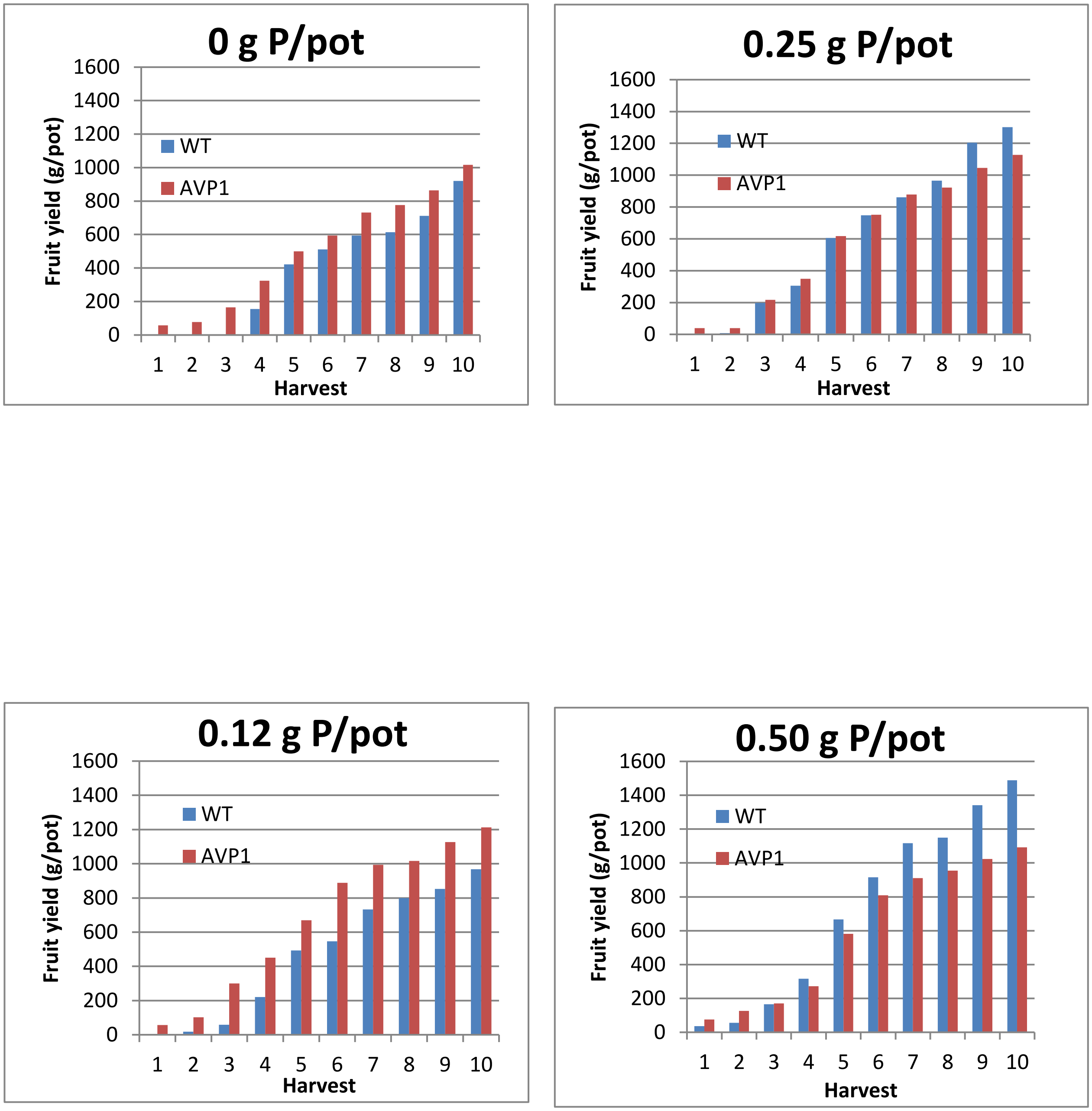

Figure 2. Cumulative fruit yield of greenhouse tomato by P rate and cultivar.

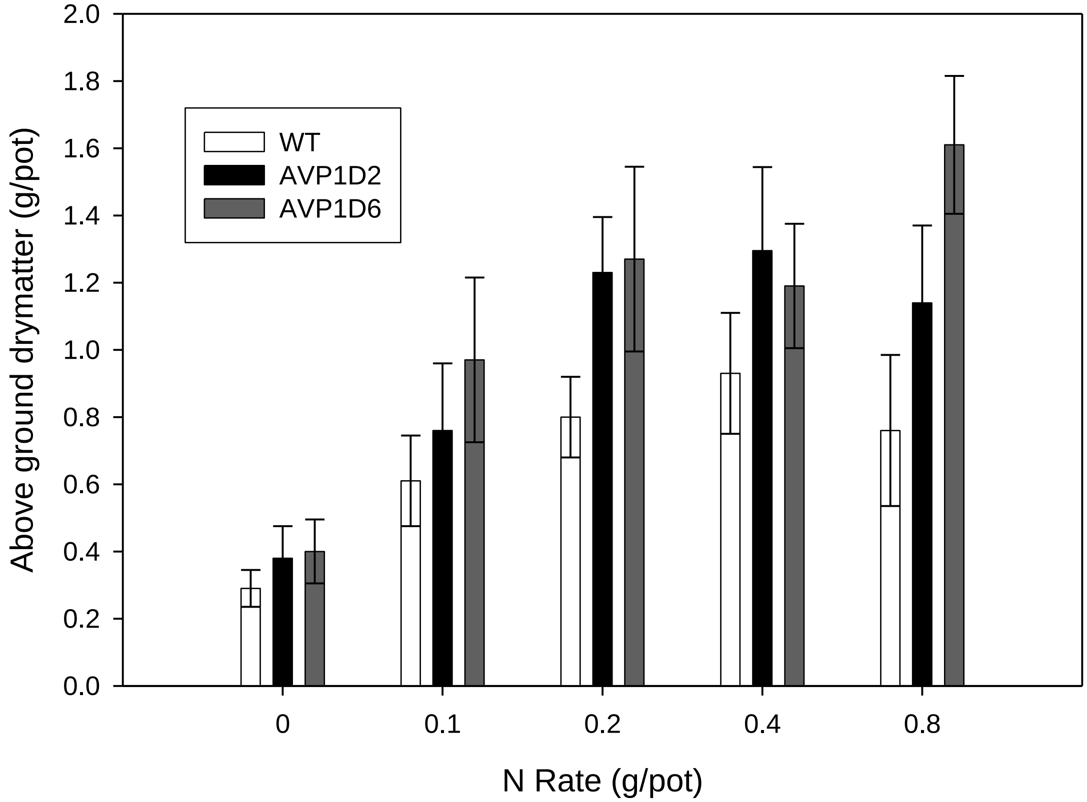

Figure 3. Dry weight of conventional and two selections (AVP1D2 and AVP1D6) of modified romaine lettuce to N.

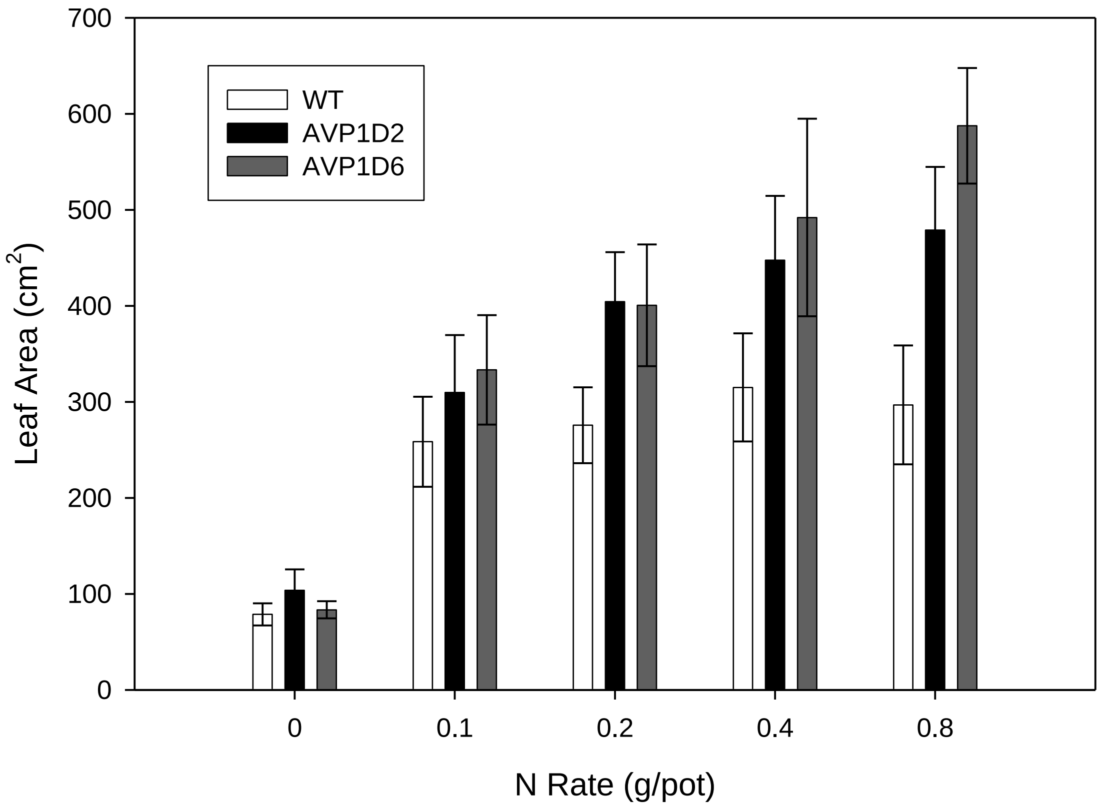

Figure 4. Leaf area of conventional and two selections (AVP1D2 and AVP1D6) of modified romaine lettuce to N.

Table 1. Total above ground N and fertilizer N (as calculated from 15N) to N rate and cultivar in greenhouse experiment.

	Conquistador	AVP1D2	AVP1D6
N Rate (g/pot)	Total Above ground N (mg/pot)		
0	3.7	5.4	5.4
0.1	22.5	25.1	28.1
0.2	33.1	44.7	49.2
0.4	47.5	59.7	51.7
0.8	33.5	46.1	63.8
Stat.	L**Q**	L**Q**	L**Q**
	Total Above ground Fertilizer N (mg/pot)		
0	-	-	-
0.1	19.7	22.0	24.5
0.2	30.9	40.8	44.9
0.4	41.4	55.6	48.4
0.8	31.4	43.3	63.8
	L**Q**	L**Q**	L**Q**

Significant linear (L) and quadratic (Q) responses to N rate at P<0.01. Cultivar effect was significant at P<0.05.

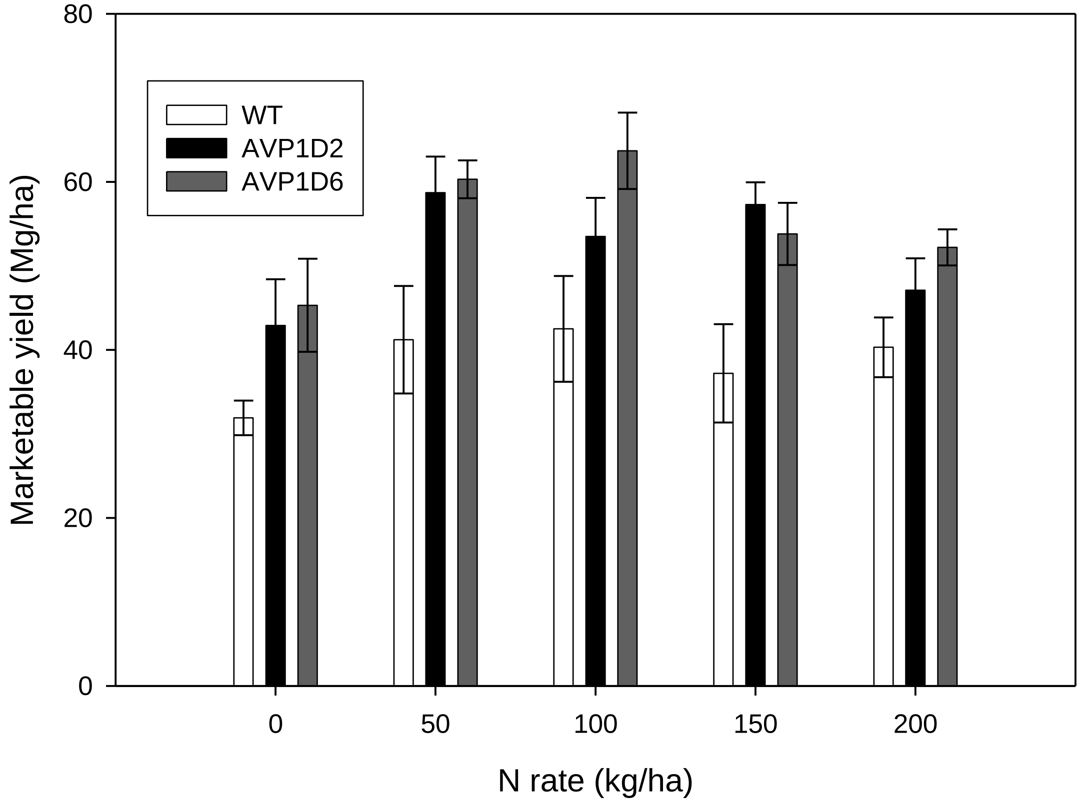

Figure 5. Marketable yield of conventional and two selections (AVP1D2 and AVP1D6) of modified romaine lettuce in a field study.

Table 2. Main effect dry matter means in greenhouse P experiments to P rate and cultivar.

Treatments	Experiment			
P rate (g/pot)	1	2	3	4
Above-ground dry matter (g/pot)				
0	1.33	0.65	0.15	0.17
0.04	2.88	1.1	0.52	1.42
0.08	2.91	1.6	0.80	1.95
0.17	3.25	1.65	0.87	2.86
0.34	3.24	2.17	0.94	3.71
	L*Q**	L**	L**Q**	L**Q*
Cultivar				
Conventional	1.74a	1.06a	0.56a	1.52a
AVP1D2	3.19b	1.77b	0.71b	2.48b
AVP1D6	3.23b	1.47ab	0.69ab	2.06ab

*,**Significant linear (L) and quadratic (Q) responses to P rate at P<0.05 and P<0.01, respectively. Cultivar effect followed by same letter were not significant at P=0.05.

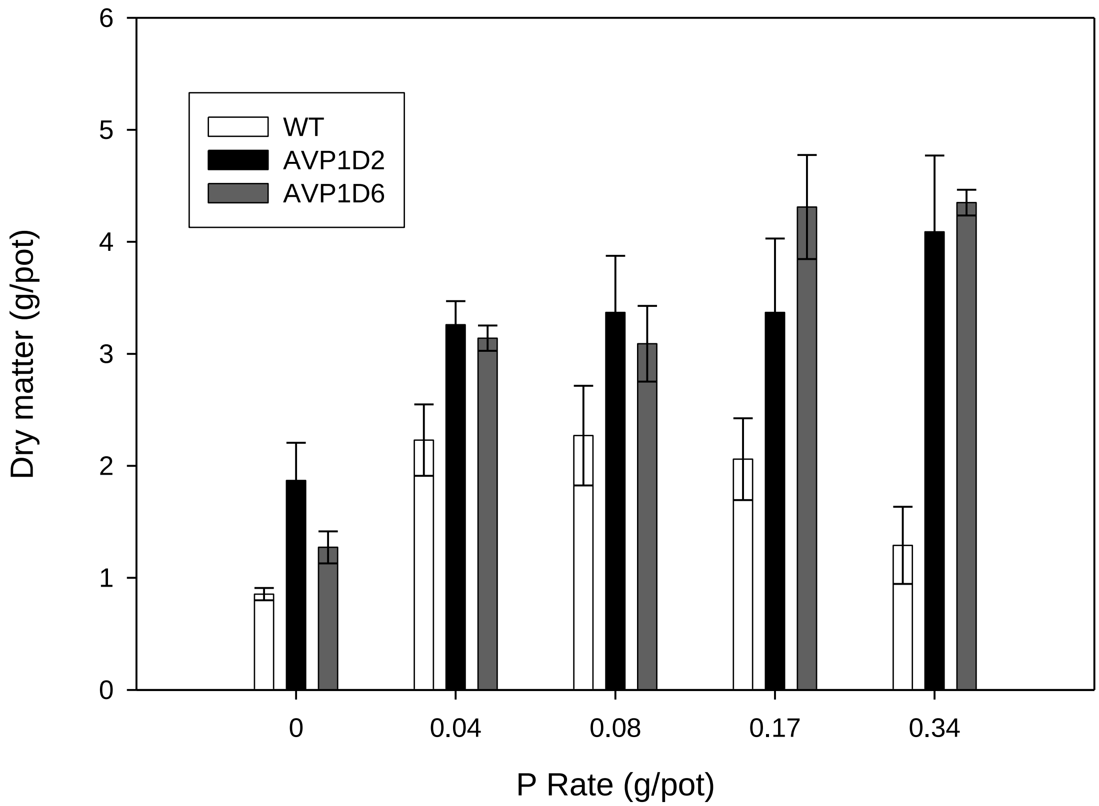

Figure 6. Dry weight of conventional and two selections (AVP1D2 and AVP1D6) of modified romaine lettuce to P fertilizer in greenhouse experiment 2.

Table 3. Main effect marketable yield means in field P experiments to P rate and cultivar.

Treatments	Experiment			
P rate (kg/ha)	1	2	3	4
Marketable yield MT/ha				
0	28.0	47.3	33.0	37.0
25	36.7	49.9	38.5	55.6
50	34.3	52.0	41.9	63.6
75	34.8	59.2	39.8	75.2
100	38.7	57.4	42.2	72.6
	L**	L**	L*	L**Q*
Cultivar				
Conventional	31.5a	47.5a	35.5a	51.9
AVP1D2	36.7b	52.2a	40.3ab	63.8
AVP1D6	35.2b	59.2b	41.4b	66.7

*,**Significant linear (L) and quadratic (Q) responses to P rate at P<0.05 and P<0.01, respectively. Cultivar effect followed by same letter were not significant at P=0.05.

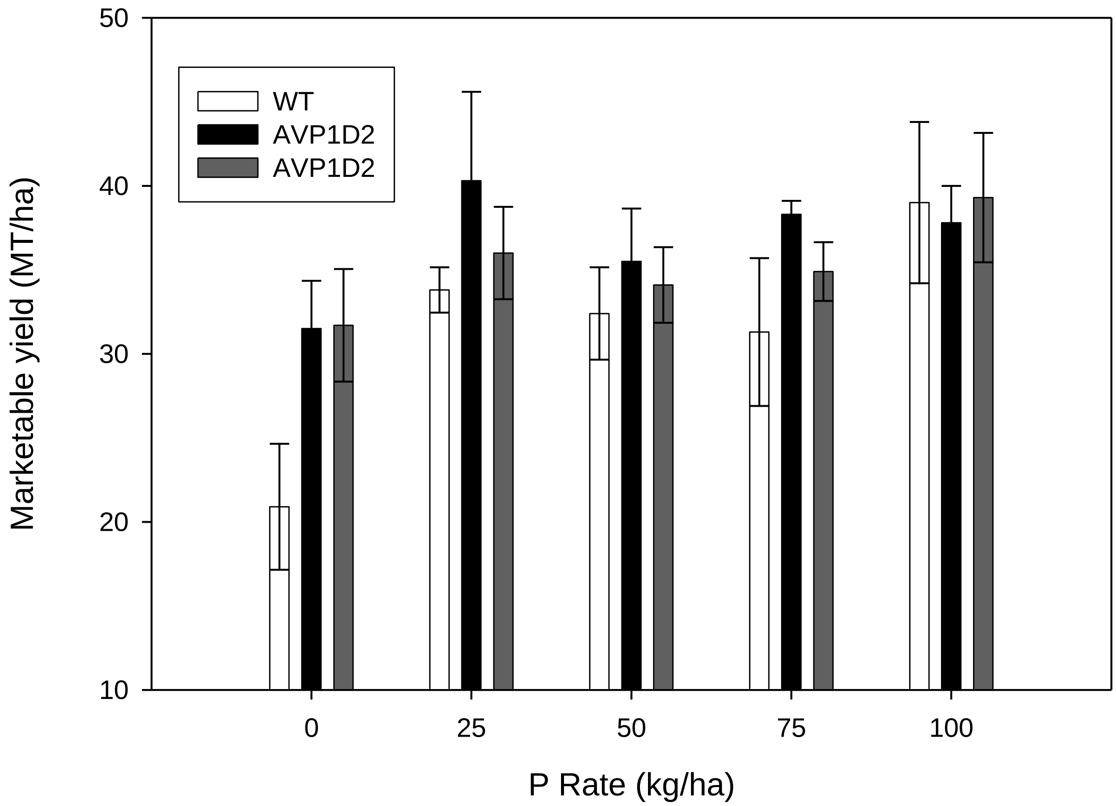

Figure 7. Marketable yield of conventional and two selections (AVP1D2 and AVP1D6) of modified romaine lettuce to P fertilizer in field experiment 1.

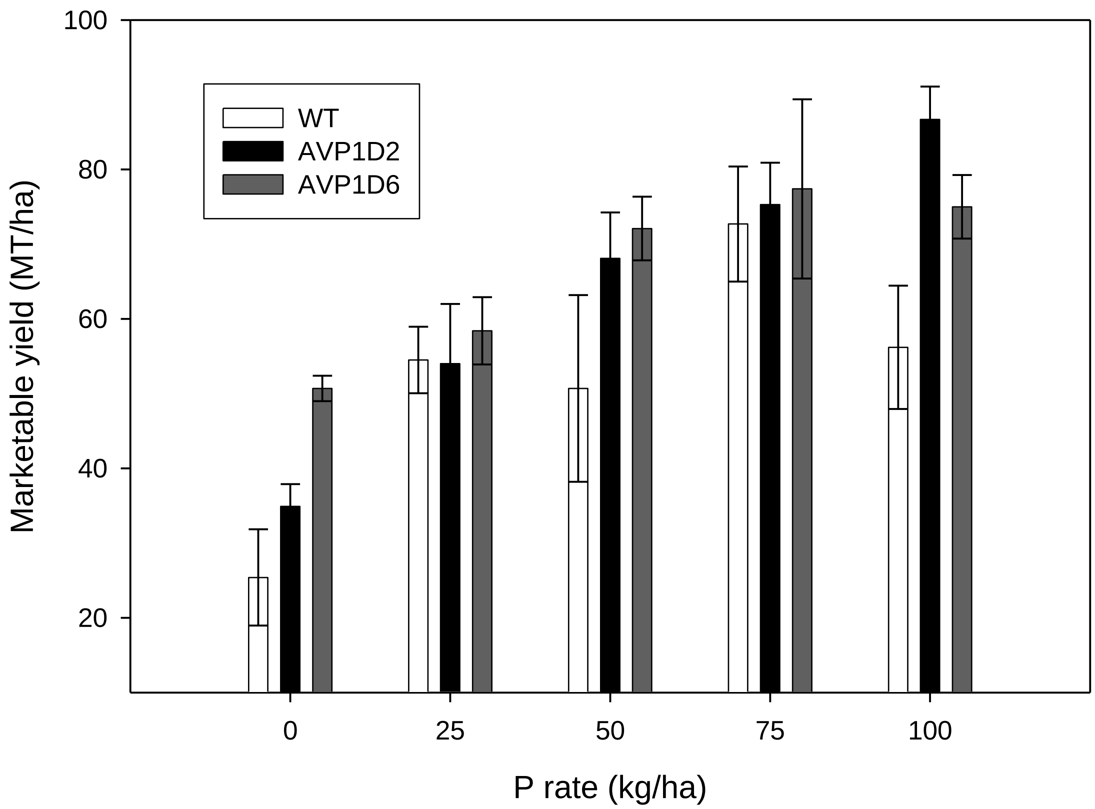

Figure 8. Marketable yield of conventional and two selections (AVP1D2 and AVP1D6) of modified romaine lettuce to P fertilizer in field experiment 4.

Table 4. Response of conventional and AVP cotton to N in 2013.

Treatment	
N Rate (kg/ha)	Yield (kg/ha)
0	1267
75	1579
150	1484
225	1676
Stat.	L**
Cultivar	
WT	1787
86	1433
68	1351
LSD	195

*,**Significant linear (L) responses to P rate at P<0.05 and P<0.01, respectively.

Table 5. Response of conventional and AVP cotton to P rate in 2013.

Treatment	
P Rate (kg/ha)	Yield (kg/ha)
0	1583
25	1711
50	1649
75	1648
Stat.	NS
Cultivar	
WT	1549
86	1456
68	2129
LSD	354

NS=not significant.

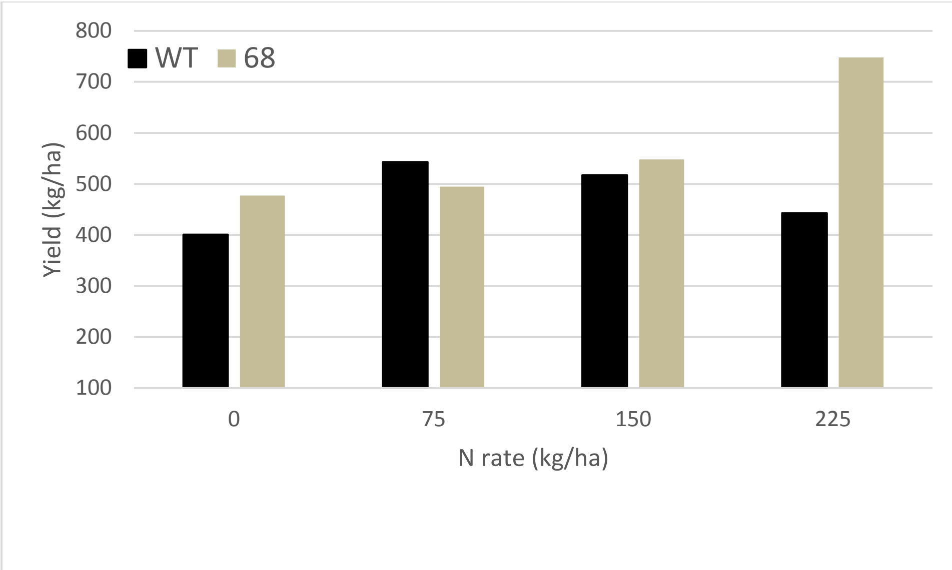

Figure 9. Response of WT and AVP cotton to N in 2014.

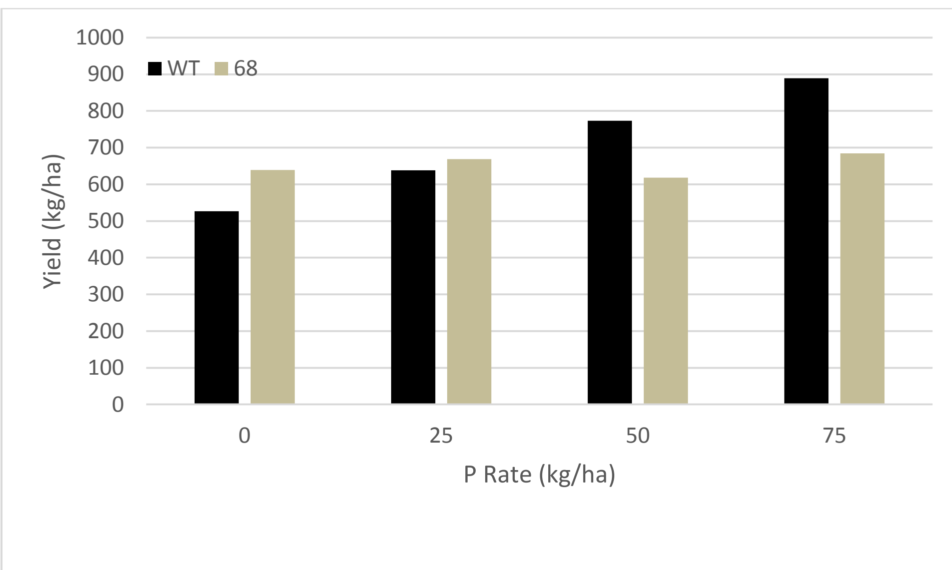

Figure 10. Response of WT and AVP cotton to N in 2014.

Table 6. Yield of conventional and AVP-OX romaine lettuce to irrigation and N fertilization in first experiment.

Treatment	Marketable Yield (MT/ha)
ET (%)	
60	43.3
120	56.5
100	60.6
N (kg/ha)	
60	50.0
120	49.7
180	57.2
Cultivar	
Conquistador	51.3
AVP1-OX	53.0
ET	**
N Rate	NS
Cultivar	NS

**Significant at the 1% level. NS=not significant.

Table 7. Yield of conventional and AVP-OX romaine lettuce to irrigation and N fertilization in 2nd experiment in 2012-2013.

Treatment	Marketable Yield (MT/ha)
ET (%)	
60	15.9
120	31.2
100	33.1
N (kg/ha)	
60	32.2
120	28.6
180	20.1
Cultivar	
Conquistador	26.9
AVP1-OX	26.8
ET	**
N Rate	**
Cultivar	**

**Significant at the 1% level. NS=not significant.

Table 8. Main effect responses of conventional and AVP-OX potato to P in 2013.

Treatment	Marketable Yield (MT/ha)
P Rate (kg/ha)	
0	11.2
50	12.4
100	11.0
Stat.	NS
Cultivar	
Desirae	13.1
Desirae AVP-OX 1	9.7
Desirae AVP-OX 2	12.1
Desirae AVP-OX 3	11.2
Stat.	NS

NS=not significant

Table 9. Main effect responses of conventional and AVP-OX tomato to P in 2013.

Treatment	Marketable Yield (MT/ha)
P Rate (kg/ha)	
0	6.0
25	6.4
50	7.1
100	6.6
	NS
Cultivar	
Money Maker	7.4
AVP-OX Money Maker	5.7
	*

*Significant at the 5% level. NS=not significant.

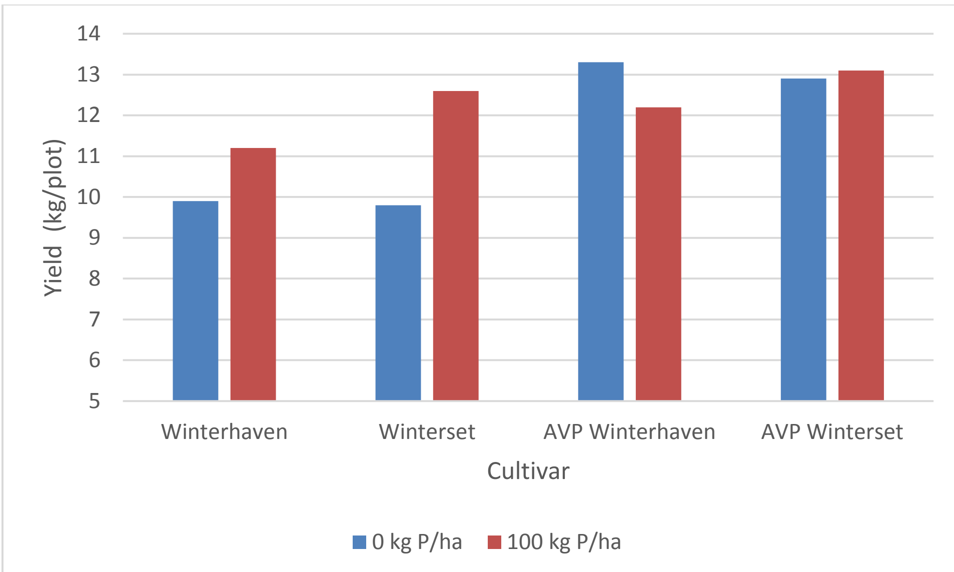

Figure 11. Response of Winterhaven. Winterset and AVP modified counterparts to P.

Table 10. Response of conventional and AVP cotton to N in 2015.

N Rate (kg/ha)	Cultivar	Yield (kg/plot)
0	Coker	225
75	Coker	195
150	Coker	250
225	Coker	211
300	Coker	262
0	AVP Coker	231
75	AVP Coker	224
150	AVP Coker	310
225	AVP Coker	307
300	AVP Coker	326
Stat. Cult		*
N Rate		L*Q*